

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
7 December 2000 (07.12.2000)

PCT

(10) International Publication Number  
**WO 00/73287 A1**

BEST AVAILABLE COPY

(51) International Patent Classification<sup>7</sup>: C07D 261/04,  
A61K 31/42

(GB). TAKLE, Andrew, Kenneth [GB/GB]; SmithKline  
Beecham Pharmaceuticals, New Frontiers Science Park  
South, Third Avenue, Harlow, Essex CM19 5AW (GB).

(21) International Application Number: PCT/EP00/04232

(22) International Filing Date: 9 May 2000 (09.05.2000)

(74) Agent: CONNELL, Anthony, Christopher; Corporate  
Intellectual Property, SmithKline Beecham, Two New  
Horizons Court, Brentford, Middlesex TW8 9EP (GB).

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
9912657.5 1 June 1999 (01.06.1999) GB

(81) Designated States (*national*): AE, AL, AM, AT, AU, AZ,  
BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK,  
DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID,  
IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT,  
LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL,  
PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ,  
UA, UG, US, UZ, VN, YU, ZA, ZW.

(71) Applicant (*for all designated States except US*):  
SMITHKLINE BEECHAM PLC [GB/GB]; New  
Horizons Court, Brentford, Middlesex TW8 9EP (GB).

(84) Designated States (*regional*): ARIPO patent (GH, GM,  
KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent  
(AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent  
(AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU,  
MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM,  
GA, GN, GW, ML, MR, NE, SN, TD, TG).

(72) Inventors; and

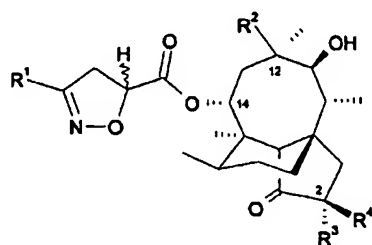
(75) Inventors/Applicants (*for US only*): DEAN, David, Ken-  
neth [GB/GB]; SmithKline Beecham Pharmaceuticals,  
New Frontiers Science Park South, Third Avenue, Harlow,  
Essex CM19 5AW (GB). HOWARD, Steven [GB/GB];  
SmithKline Beecham Pharmaceuticals, New Frontiers  
Science Park South, Third Avenue, Harlow, Essex CM19  
5AW (GB). NAYLOR, Antoinette [GB/GB]; SmithK-  
line Beecham Pharmaceuticals, New Frontiers Science  
Park South, Third Avenue, Harlow, Essex CM19 5AW

Published:

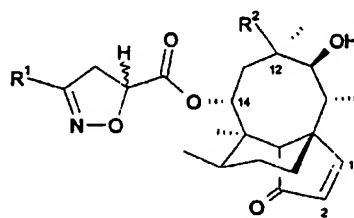
— With international search report.

For two-letter codes and other abbreviations, refer to the "Guid-  
ance Notes on Codes and Abbreviations" appearing at the begin-  
ning of each regular issue of the PCT Gazette.

(54) Title: ISOXAZOLINE CARBOXYLATE DERIVATIVES OF MUTILINE AND THEIR USE AS ANTIBACTERIALS



(IA)



(IB)

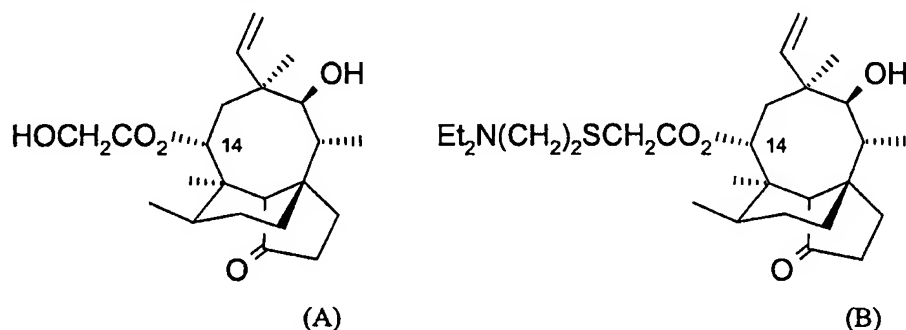
(57) Abstract: Pleuromutinin derivatives of the formulae (IA) and (IB) have potent anti-microbial activity and are of use in anti-infective therapy.

WO 00/73287 A1

## ISOXAZOLINE CARBOXYLATE DERIVATIVES OF MUTILINE AND THEIR USE AS ANTIBACTERIALS

The present invention relates to novel compounds, to processes for their preparation, to pharmaceutical compositions containing them and to their use in medical therapy, particularly  
 5 antibacterial therapy.

Pleuromutilin, the compound of formula (A), is a naturally occurring antibiotic which has anti-mycoplasmal activity and modest antibacterial activity. Mutilin and other compounds with a free OH at C-14 are inactive. The impact of further modification at C-14 on the activity of  
 10 pleuromutilin has been investigated (H. Egger and H. Reinshagen, *J. Antibiotics*, 1976, 29, 923). Replacing the hydroxy group of the glycolic ester moiety at position 14 by another O, S or N-linked group was found to improve anti-microbial activity. Thus, introducing a diethylaminoethylthio group gives the compound of formula (B), also known as Tiamulin, which is used as a veterinary antibiotic (G. Hogenauer in *Antibiotics*, Vol. V, part 1, ed.  
 15 F.E. Hahn, Springer-Verlag, 1979, p.344).



In this application, the non-conventional numbering system which is generally used in the literature (G. Hogenauer, loc. cit.) is used.

20

WO 97/25309 (SmithKline Beecham) describes further modification of the acyloxy group, disclosing 14-*O*-carbamoyl derivatives of mutilin or 19,20-dihydromutilin, in which the N-atom of the carbamoyl group is unsubstituted, mono- or di-substituted.

25

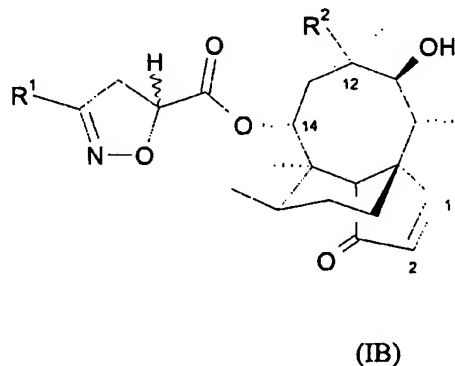
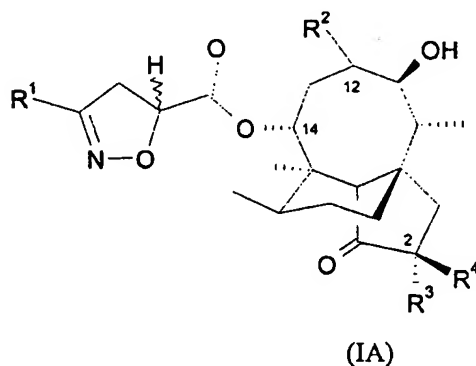
WO 98/05659 (SmithKline Beecham) discloses 14-*O*-carbamoyl derivatives of mutilin or 19,20-dihydromutilin, in which the N-atom of the carbamoyl group is acylated by a group which includes an azabicyclic moiety.

30

WO 99/21855 (SmithKline Beecham) describes further derivatives of mutilin or 19,20-dihydromutilin, in which the glycolic ester moiety at position 14 is modified.

The present invention is based on the unexpected discovery that novel mutilin derivatives having an isoxazoline carboxylate substituent at the 14-position also have potent antimicrobial activity.

- 5 Accordingly the present invention provides a compound of formula (IA) or (IB):



in which:

- 10  $R^1$  is an optionally substituted aryl group, or an optionally substituted nitrogen containing heterocycle;

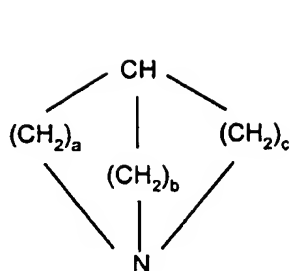
$R^2$  is vinyl or ethyl;

$R^3$  is H, OH or F; and  $R^4$  is H, or  $R^3$  is H and  $R^4$  is F.

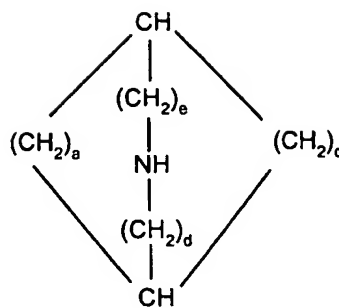
- 15 Representative examples of  $R^1$  when an aryl group include phenyl. When  $R^1$  is aryl, a preferred number of substituents is up to three, more preferred is one. Representative substituents include (C<sub>1-6</sub>)alkoxy, for example methoxy.

Preferably  $R^1$  is a nitrogen containing heterocycle.

- 20 When used herein the term "nitrogen containing heterocycle" refers to a saturated or partially saturated non-aromatic mono- or bicyclic group linked via a ring carbon atom. The group may comprise one to three nitrogen atoms, preferably one or two, more preferably one nitrogen atom. When the group is monocyclic it can contain between 4 and 8 ring atoms, and when bicyclic it can contain between 5 and 10 ring atoms in each ring. The aza-bicyclic ring system  
25 may, for example, be represented by the formulae (X) or (Y):



(X)



(Y)

wherein each of a, b, c, d and e, which may be the same or different, is for a, b and c an integer from 1 to 4, and for d and e 0 or an integer from 1 to 3, such that any one ring has between 5 and 10 ring atoms.

The nitrogen containing heterocycle can be optionally substituted on carbon by up to 3 substituents. Suitable substituents include alkyl, alkyloxy, alkenyl and alkenyloxy, each of which may be carried by either a bridgehead or a non-bridgehead carbon atom. In addition, the or each nitrogen atom may be substituted by oxygen, to form an N-oxide, or by mono- or di(C<sub>1-6</sub>)alkyl, in which case it will be appreciated that a quaternary cation can be formed. Representative nitrogen substituents include (C<sub>1-6</sub>) alkyl, preferably methyl. The counter ion may be a halide ion such as chloride or bromide, preferably chloride. The ring system additionally may contain one or more double bonds.

Representative nitrogen containing heterocycles include optionally substituted azabicyclo-octyl and piperidinyl. Preferred nitrogen containing heterocyclic moieties include optionally substituted 1-aza-bicyclo[2.2.2]oct-4-yl, piperidin-4-yl and 8-aza-bicyclo[3.2.1]oct-3-yl. The linking ring carbon in an azabicyclic system may be a bridgehead atom or a non-bridgehead atom.

When used herein, the term "aryl" refers to, unless otherwise defined, phenyl or naphthyl optionally substituted with up to five, preferably up to three substituents.

Suitable substituents for an aryl group include, for example, and unless otherwise defined, halogen, (C<sub>1-6</sub>)alkyl, aryl, aryl(C<sub>1-6</sub>)alkyl, (C<sub>1-6</sub>)alkoxy, (C<sub>1-6</sub>)alkoxy(C<sub>1-6</sub>)alkyl, halo(C<sub>1-6</sub>)alkyl, aryl(C<sub>1-6</sub>)alkoxy, hydroxy, nitro, cyano, azido, amino, mono- and di-N-(C<sub>1-6</sub>)alkylamino, acylamino, arylcarbonylamino, acyloxy, carboxy, carboxy salts, carboxy

esters, carbamoyl, mono- and di-*N*-(C<sub>1-6</sub>)alkylcarbamoyl, (C<sub>1-6</sub>)alkoxycarbonyl, aryloxycarbonyl, ureido, guanidino, (C<sub>1-6</sub>)alkylguanidino, amidino, (C<sub>1-6</sub>)alkylamidino, sulphonylamino, aminosulphonyl, (C<sub>1-6</sub>)alkylthio, (C<sub>1-6</sub>)alkylsulphinyl, (C<sub>1-6</sub>)alkylsulphonyl, heterocyclyl, heteroaryl, heterocyclyl(C<sub>1-6</sub>)alkyl and  
5 heteroaryl(C<sub>1-6</sub>)alkyl. In addition, two adjacent ring carbon atoms may be linked by a (C<sub>3-5</sub>)alkylene chain, to form a carbocyclic ring.

When used herein, the terms "alkyl" and "alkenyl" refer to (individually or as part of alkoxy or alkenyloxy) straight and branched groups containing up to six carbon atoms and are optionally  
10 substituted by one or more groups selected from the group consisting of aryl, heteroaryl, heterocyclyl, (C<sub>1-6</sub>)alkoxy, (C<sub>1-6</sub>)alkylthio, aryl(C<sub>1-6</sub>)alkoxy, aryl(C<sub>1-6</sub>)alkylthio, amino, mono- or di-(C<sub>1-6</sub>)alkylamino, cycloalkyl, cycloalkenyl, carboxy and esters thereof, amide, ureido, guanidino, (C<sub>1-6</sub>)alkylguanidino, amidino, (C<sub>1-6</sub>)alkylamidino, (C<sub>1-6</sub>)acyloxy, azido, hydroxy, and halogen.

15 When used herein, the terms "cycloalkyl" and "cycloalkenyl" refer to groups having from three to eight ring carbon atoms and are optionally substituted as described hereinabove for alkyl and alkenyl groups.

20 When used herein the terms "heterocyclyl" and "heterocyclic" refer to, unless otherwise defined, non-aromatic, single and fused, rings suitably containing up to four heteroatoms in each ring, each of which is selected from oxygen, nitrogen and sulphur, which rings, may be unsubstituted or substituted by, for example, up to three substituents. Each heterocyclic ring preferably has from 4 to 7, preferably 5 or 6, ring atoms. A fused heterocyclic ring system  
25 may include carbocyclic rings and need include only one heterocyclic ring.

Preferably a substituent for a heterocyclyl group is selected from oxo, and the group hereinbefore defined as suitable aryl substituents.

30 When used herein, the term "heteroaryl" suitably includes, unless otherwise defined, a mono- or bicyclic heteroaromatic ring system comprising up to four, preferably 1 or 2, heteroatoms each selected from oxygen, nitrogen and sulphur. Each ring may have from 4 to 7, preferably 5 or 6, ring atoms. A bicyclic heteroaromatic ring system may include a carbocyclic ring.

Preferably a substituent for a heteroaromatic ring system is selected from the group hereinbefore defined as suitable aryl substituents.

Depending on the substituents, two or more diastereoisomers may be possible. In that situation the present invention includes the individual diastereoisomers and mixtures thereof.

5

The 2-hydroxy-substituted compounds of formula (IA) are of the (2*S*) configuration. The 2-F-substituted compounds of formula (IA) may of (2*S*) configuration or (2*R*) configuration, or be provided as mixtures thereof. The (2*S*) configuration is however preferred.

- 10 The stereochemistry at the 5-position in the isoxazoline ring may be either of *R* configuration or *S* configuration, or a mixture thereof.

Preferred compounds of the invention include:

- 15 19,20-Dihydromutilin-14-[(5*R,S*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate];  
 19,20-Dihydromutilin-14-[(5*R*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate];  
 19,20-Dihydromutilin-14-[(5*S*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate];  
 20 Mutilin-14-[(5*R,S*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate];  
 Mutilin-14-[(5*S*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate];  
 Mutilin-14-[(5*R*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate];  
 Mutilin-14-[(5*R,S*)-3-(1-methyl-piperidin-4-yl)-4,5-dihydroisoxazole-5-carboxylate], and  
 25 Mutilin-14-[(5*R,S*)-3-(*exo*-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-4,5-dihydroisoxazole-5-carboxylate].

The compounds of this invention may be in crystalline or non-crystalline form, and, if crystalline, may optionally be hydrated or solvated. This invention includes within its scope stoichiometric hydrates as well as compounds containing variable amounts of water.

30

The compounds according to the invention are suitably provided in substantially pure form, for example at least 50% pure, suitable at least 60% pure, advantageously at least 75% pure, preferably at least 85% pure, more preferably at least 95% pure, especially at least 98% pure, all percentages being calculated as weight/weight.

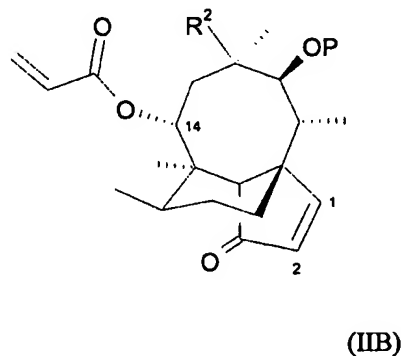
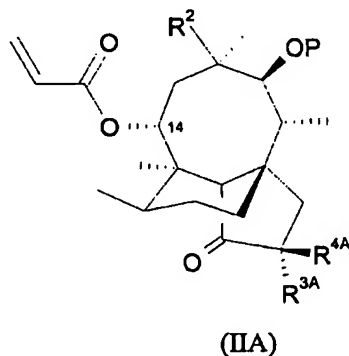
Compounds of the invention that contain a basic group such as an amino substituent may be in the form of a free base or an acid addition salt. Compounds having an acidic group such as a carboxy substituent may be in the form of a pharmaceutically acceptable salt. Compounds of the invention having both a basic and an acidic centre may be in the form of zwitterions, acid addition salt of the basic centre or alkali metal salts (of the carboxy group). Pharmaceutically acceptable salts are preferred.

Pharmaceutically acceptable acid-addition salts include those described by Berge, Bighley, and Monkhouse, *J. Pharm. Sci.*, 1977, 66, 1-19. Suitable salts include the hydrochloride, maleate, and methanesulphonate; particularly the hydrochloride.

Pharmaceutically acceptable salts include those described by Berge, Bighley, and Monkhouse, *J. Pharm. Sci.*, 1977, 66, 1-19. Suitable salts include alkali metal salts such as the sodium and potassium salts.

Compounds of the present invention may be readily prepared from a pleuromutilin or a 19,20-dihydro-pleuromutilin derivative by adapting procedures well known in the art for forming an isoxazoline group. Suitable procedures are reviewed, for example, by W. Curruthers in *Cycloaddition Reactions in Organic Synthesis*, Tetrahedron Organic Chemistry Series, vol. 8; J.E. Baldwin and P.D. Magnus, eds., (Pergamon Press).

Accordingly, the present invention provides a process for preparing a compound of formula (IA) or (IB) which comprises reacting a compound of formula (IIA) or (IIB):

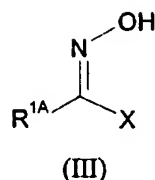


in which:

P is hydrogen or a removable hydroxy-protecting group;

$R^{3A}$  and  $R^{4A}$  are  $R^3$  and  $R^4$  as defined for formulae (IA) and (IB) or a group convertible to  $R^3$  and  $R^4$  respectively; and  
 $R^2$  is as hereinbefore defined;

- 5 with a compound of formula (III):



in which:

- $R^{1A}$  is  $R^1$  as defined for formulae (IA) and (IB) or a group convertible to  $R^1$ ; and  
 10 X is chloro or bromo, or a group convertible to chloro or bromo;  
 in a 1,3-dipolar cycloaddition reaction and thereafter, and if so needed;  
 converting P to hydrogen, and if necessary  
 converting an  $R^{3A}$  or  $R^{4A}$  group to an  $R^3$  or  $R^4$  group.  
 15 Suitable ring forming conditions are well known in the art (*e.g.* *J. Org. Chem.*, 1968, 33(1), 476-478) and include an organic solvent such as N,N-dimethylformamide, dichloromethane or 1,4-dioxane (preferably N,N-dimethylformamide or a mixture of dichloromethane and 1,4-dioxane), at a temperature of  $-20^{\circ}\text{C}$  to  $60^{\circ}\text{C}$  (preferably  $-10^{\circ}\text{C}$  to  $25^{\circ}\text{C}$ ), and in the presence of a tertiary organic base such as triethylamine, di-isopropyl ethylamine or N-methylmorpholine (preferably triethylamine).  
 20

It will be appreciated that this ring forming process generally produces a mixture of *R* and *S* isomers at the 5 position of the isoxazoline ring. These may be separated by conventional techniques such as chromatography or fractional crystallisation.

25

Conversion of an  $R^{1A}$ ,  $R^{3A}$  or  $R^{4A}$  group to an  $R^1$ ,  $R^3$  or  $R^4$  group typically arises when a protecting group is needed during the above ring-forming reaction or during the preparation of the reactants by the procedures described below

- 30 Preferably P is a hydroxyl protecting group such as an acyl group, for example so that -OP is trifluoroacetoxy or dichloroacetoxy. When the intended  $R^3$  is also hydroxyl, then  $R^{3A}$  is also



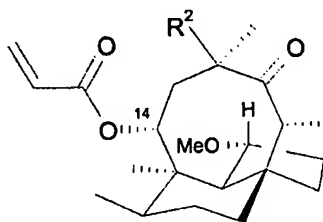
preferably acyloxy, for example acetyl or dichloroacetyl. Hydroxyl groups at positions 11 and 2 (as groups OP and R<sup>3A</sup>) may be protected using, for example, trifluoroacetic anhydride or dichloroacetic anhydride and pyridine in tetrahydrofuran or *N*-trifluoroacetyl-imidazole in tetrahydrofuran at 0°C. After the reaction described above with (III) is complete, the protecting acyl groups may be removed to restore the hydroxyl groups, for instance by hydrolysis *e.g.* using NaOH in either MeOH or tetrahydrofuran/water solution.

Suitable hydroxy, carboxy and amino protecting groups are those well known in the art and which may be removed under conventional conditions and without disrupting the remainder of the molecule. A comprehensive discussion of the ways in which hydroxy, carboxy and amino groups may be protected and methods for cleaving the resulting protected derivatives is given in for example *Protective Groups in Organic Chemistry*, T.W. Greene and P.G.M. Wuts, (Wiley-Interscience, New York, 2nd edition, 1991). Particularly suitable hydroxy protecting groups include, for example, triorganosilyl groups such as, for instance, trialkylsilyl and also organocarbonyl and organooxycarbonyl groups such as, for instance, acetyl, allyloxycarbonyl and 4-methoxybenzyloxycarbonyl. Particularly suitable carboxy protecting groups include alkyl and aryl esters, for instance methyl, ethyl and phenyl. Particularly suitable amino protecting groups include alkoxycarbonyl and 4-methoxybenzyloxycarbonyl.

R<sup>3A</sup> is typically hydrogen, fluoro or protected hydroxyl, such as acyloxy. After the coupling reaction, protecting acyl groups may be removed to restore the hydroxyl groups by hydrolysis *e.g.* using NaOH in MeOH.

A compound of formula (IA) may also be prepared from an *epi*-mutilin starting material.

Accordingly, in a further aspect, the present invention provides a process for preparing a compound of formula (IA) in which R<sup>3</sup> and R<sup>4</sup> are both hydrogen which comprises reacting an *epi*-mutilin compound of formula (IIC):



(IIC) wherein  $R^2$  is as hereinbefore defined;

with a compound (III), as hereinbefore defined;

and then treating the product with an acid;

and where required or desired converting an  $R^{1A}$  group to an  $R^1$  group.

5

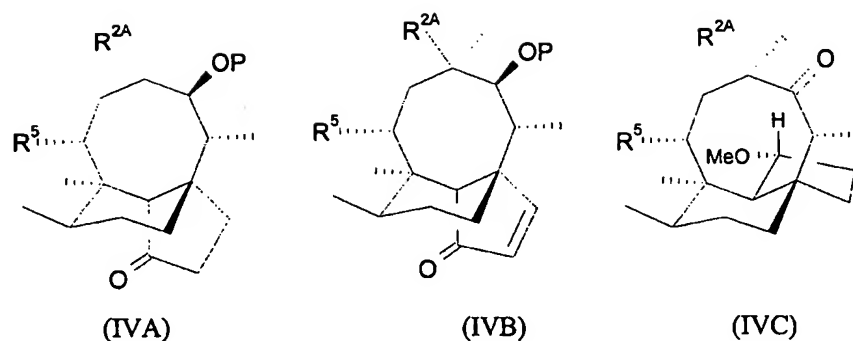
The acid treatment indicated above converts the *epi*-mutilin configuration of formula (IIC) to the usual mutilin nucleus of formula (IIA). Typically this conversion is carried out by treatment with conc. HCl or Lukas reagent (conc. HCl saturated with  $ZnCl_2$ ) in dioxane.

- 10 It should be appreciated that it may be necessary to interconvert one  $R^1$ ,  $R^2$ ,  $R^3$  or  $R^4$  group to another  $R^1$ ,  $R^2$ ,  $R^3$  or  $R^4$  group. This typically arises when one compound of formula (IA/B) is used as the immediate precursor of another compound of formula (IA/B) or when it is easier to introduce a more complex or reactive substituent at the end of a synthetic sequence.

- A substituent group in  $R^1$  can be converted into another substituent group using one of the  
15 general methods for functional group transformation described in the literature (e.g. a carboxylic ester can be hydrolysed to a carboxylic acid with base; an acid can be converted into an amide; a *tert*-butoxy-carbonyl-amino group can be converted into an amine by treatment with trifluoroacetic acid; an amino group can be alkylated or acylated), provided that the method chosen is compatible with other functional groups in the molecule (e.g. the ketone  
20 at C-3 in the pleuromutillin nucleus, the isoxazoline or the acrylate groups).

- Functional group transformations are well known in the art and are described in, for instance, *Comprehensive Organic Functional Group Transformations*, eds. A.R. Katritzky, O. Meth-Cohn, and C.W. Rees (Elsevier Science Ltd., Oxford, 1995), *Comprehensive Organic*  
25 *Chemistry*, eds. D. Barton and W.D. Ollis (Pergamon Press, Oxford, 1979), and *Comprehensive Organic Transformations*, R.C. Larock (VCH Publishers Inc., New York, 1989).

- Compounds of formulae (IIA) in which  $R^{3A}$  and  $R^{4A}$  are hydrogen, (IIB) and (IIC) may be  
30 readily prepared by reacting a compound of formulae (IVA), (IVB), or (IVC):



in which  $R^5$  is hydroxy, or a group convertible to hydroxy; P is as hereinbefore defined; and  $R^{2A}$  is  $R^2$  as hereinbefore defined, or groups convertible to  $R^2$ ;

- 5 with acryloyl chloride in a solvent such as dichloromethane in the presence of an organic base such as triethylamine;  
and thereafter, and if so needed;  
converting P to hydrogen,  
and/or converting the *epi*-mutilin configuration of formula (IVC) to the usual mutilin nucleus  
10 of formula (IVA) by treatment with conc. HCl or Lukas reagent as described above.

- $R^{2A}$  is typically the  $R^2$  group vinyl, and this may be converted to the alternative  $R^2$  ethyl group by hydrogenating the vinyl group to form an ethyl group, typically by hydrogenation over a palladium catalyst (*e.g.* 10% palladium-on-carbon) in a solvent such as ethyl acetate,  
15 ethanol, dioxane, or tetrahydrofuran. The skilled person will appreciate that the conversion of  $R^{2A}$  to  $R^2$  may not be feasible after the formation of the acrylate group, the isoxazoline ring or compounds of formula (IVB), thus such modifications should be carried out prior to these synthetic modifications.

- 20 Compounds of formula (IIA) in which  $R^{3A}$  is hydroxyl or fluoro may be prepared from pleuromutilin, via an intermediate 2-diazo compound, the preparation of which is described by G. Schulz and H. Berner in *Tetrahedron*, 1984, 40, 905. Where necessary, saponification of the C14 ester group may be carried out at an appropriate stage using conventional techniques such as sodium hydroxide or sodium methoxide in methanol or aqueous tetrahydrofuran  
25 solution. Treatment with acryloyl chloride as hereinbefore described provides a compound of formula (IIA).

The intermediate 2-diazo compound may be reacted with a carboxylic acid to give a 2-acyloxy-mutilin derivative. Suitably, reaction with dichloroacetic acid gives a 2-dichloroacetoxymutilin derivative, which can be deprotected as described above to provide the (2S)-2-hydroxy derivative, at an appropriate stage.

5

Compounds of formula (IIA) in which R<sup>3A</sup> is fluoro may be obtained by reacting 2-diazomutilin with a source of hydrogen fluoride. Conveniently, the hydrogen fluoride source is an amine complex of hydrogen fluoride such as hydrogen fluoride-pyridine. The reaction may be carried out in an anhydrous solvent (e.g. diethyl ether, tetrahydrofuran, 1,2-dimethoxyethane),  
10 at a temperature of -15°C to 25°C. This reaction produces (2S)-2-fluoro derivatives. (2R)-2-Fluoro-mutilin derivatives may be prepared by treating the (2S)-isomer with a base (e.g. sodium hydroxide or potassium hydroxide in ethanol). This will usually produce a mixture of (2S) and (2R)-isomers that may be separated using conventional techniques such as chromatography and crystallisation.

15

Compounds of formula (IV) may be readily prepared according to methods described in the literature, for example G. Schulz and H. Berner, *Tetrahedron*, 1984, 40, 905, and in WO 97/25309 and WO 98/05659 (SmithKline Beecham). Where necessary, and as hereinbefore described, saponification of the C14 ester may be carried out at an appropriate stage.

20

Compounds of formula (III) may be readily prepared by adapting procedures well known in the art for preparing  $\alpha$ -haloaloximes. Suitable references include *Comprehensive Organic Chemistry*, 1991, vol. 4, 1078, B.M. Trost and I. Fleming, eds. (Pergamon Press, Oxford). Accordingly the present invention provides a process for forming a compound of formula (III)  
25 which comprises reacting a suitable aldehyde with hydroxylamine hydrochloride in a solvent such as methanol at 0°C to 25°C. The resulting aldoxime may be reacted with a suitable halogenating agent such as N-bromosuccinimide, N-chlorosuccinimide or chlorine in a solvent such as N,N-dimethylformamide, pyridine or carbon tetrachloride to give an  $\alpha$ -haloaldoxime of formula (III).

30

Suitable aldehydes may be readily prepared by adapting procedures well known in the art and are described in, for instance, *Comprehensive Functional Group Transformations*, Vol 3, A.R. Katritzky, O. Meth-Kohn and C.W. Rees eds. (Elsevier Science Ltd., Oxford, 1995).

Alternatively the present invention provides for the *in situ* generation of compounds of formula (III) which comprises reacting an aldoxime with a halogenating agent such as N-bromosuccinimide in a solvent such as N,N-dimethylformamide at 0°C to 25°C. The compound of formula (IIA) or (IIB) may then be introduced to the reaction mixture containing  
5 the  $\alpha$ -haloaldoxime of formula (III) at a temperature of -10°C in the presence of an organic base such as triethylamine.

The compounds of the present invention may contain a chiral centre, and therefore the above processes may produce a mixture of diastereoisomers. A single diastereoisomer may be  
10 prepared by separating such a mixture of diastereoisomers by conventional techniques such as chromatography or fractional crystallisation.

The compounds of this invention may be in crystalline or non-crystalline form, and, if crystalline, may optionally be hydrated or solvated. When some of the compounds of this  
15 invention are allowed to crystallise or are recrystallised from organic solvents, solvent of crystallisation may be present in the crystalline product. Similarly, some of the compounds of this invention may be crystallised or recrystallised from solvents containing water. In such cases water of hydration may be present in the crystalline product. Crystallisation procedures will usually produce stoichiometric hydrates. Compounds containing variable amounts of  
20 water may be produced by processes such as lyophilisation.

The compounds according to the invention are suitably provided in substantially pure form, for example at least 50% pure, suitable at least 60% pure, advantageously at least 75% pure, preferably at least 85% pure, more preferably at least 95% pure, especially at least 98% pure,  
25 all percentages being calculated as weight/weight. An impure or less pure form of a compound according to the invention may, for example, be used in the preparation of a more pure form of the same compound or of a related compound (for example a corresponding derivative) suitable for pharmaceutical use.

30 The present invention also includes pharmaceutically acceptable salts and derivatives of the compounds of the invention. Salt formation may be possible when one of the substituents carries an acidic or basic group. Salts may be prepared by salt exchange in conventional manner.

Acid-addition salts may be pharmaceutically acceptable or non-pharmaceutically acceptable. In the latter case, such salts may be useful for isolation and purification of the compound of the invention, or intermediates thereto, and will subsequently be converted into a pharmaceutically acceptable salt or the free base.

5

The compounds of the present invention and their pharmaceutically acceptable salts or derivatives have antimicrobial properties and are therefore of use in therapy, in particular for treating microbial infections in animals, especially mammals, including humans, in particular humans and domesticated animals (including farm animals). The compounds may be used for the treatment of infections caused by, for example, Gram-positive and Gram-negative bacteria and mycoplasmas, including, for example, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Haemophilus sp.*, *Neisseria sp.*, *Legionella sp.*, *Chlamydia sp.*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, and *Mycoplasma gallisepticum*.

15

The present invention also provides a method of treating microbial infections in animals, especially in humans and in domesticated mammals, which comprises administering a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof, or a composition according to the invention, to a patient in need thereof.

20

The invention further provides the use of a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof in the preparation of a medicament for use in the treatment of microbial infections.

25

Compounds of the present invention may be used to treat skin and soft tissue infections and acne, by topical application. Accordingly, in a further aspect the present invention provides the use of a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof in the preparation of a medicament adapted for topical administration for use in the treatment of skin and soft tissue infections and also in the treatment of acne in humans.

30

Compounds of the present invention may be also used for the elimination or reduction of nasal carriage of pathogenic bacteria such as *S. aureus*, *H. influenzae*, *S. pneumonia* and *M. catarrhalis*, in particular colonisation of the nasopharynx by such organisms, by the administration of a compound of the present invention thereto. Accordingly, in a further

aspect, the present invention provides for the use of a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof in the manufacture of a medicament adapted for administration to the nasal cavity, for reducing or eliminating the nasal carriage of pathogenic organisms. Preferably, the medicament is adapted for focussed  
5 delivery to the nasopharynx, in particular the anterior nasopharynx.

Such reduction or elimination of nasal carriage is believed to be useful in prophylaxis of recurrent acute bacterial sinusitis or recurrent otitis media in humans, in particular in reducing the number of episodes experienced by a patient over a given period of time or increasing the  
10 time intervals between episodes. Accordingly, in a further aspect, the present invention provides for the use of a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof in the manufacture of a medicament adapted for administration to the nasal cavity, for prophylaxis of recurrent acute bacterial sinusitis or recurrent otitis media.

15 Compounds of the present invention are also useful in treating chronic sinusitis. Accordingly, in a further aspect, the present invention provides for the use of a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof in the manufacture of a medicament, for treating of chronic sinusitis.

20 The compounds according to the invention may suitably be administered to the patient at a daily dosage of from 1.0 to 50 mg/kg of body weight. For an adult human (of approximately 70 kg body weight), from 50 to 3000 mg, for example about 1500 mg, of a compound according to the invention may be administered daily. Suitably, the dosage for adult humans is from 5 to 20 mg/kg per day. Higher or lower dosages may, however, be used in accordance  
25 with normal clinical practice.

To lessen the risk of encouraging the development of resistant organisms during prophylaxis of recurrent otitis media or recurrent acute bacterial sinusitis, it is preferred to administer the drug on an intermittent, rather than a continual, basis. In a suitable intermittent treatment  
30 regimen for prophylaxis of recurrent otitis media or recurrent sinusitis, drug substance is administered on a daily basis, for a small number of days, for instance from 2 to 10, suitably 3 to 8, more suitably about 5 days, the administration then being repeated after an interval, for instance, on a monthly basis over a period of months, for instance up to six months. Less preferably, the drug substance may be administered on a continuing, daily basis, over a

prolonged period, for instance several months. Suitably, for prophylaxis of recurrent otitis media or recurrent sinusitis, drug substance is administered once or twice a day. Suitably, drug substance is administered during the winter months when bacterial infections such as recurrent otitis media and recurrent sinusitis tend to be more prevalent. The drug substance  
5 may be administered at a dosage of from 0.05 to 1.00mg, typically about 0.1 to 0.2mg, in each nostril, once or twice a day.

More generally, the compounds and compositions according to the invention may be formulated for administration in any convenient way for use in human or veterinary medicine,  
10 by analogy with other antibiotics.

Accordingly, in a further aspect, the present invention provides a pharmaceutical composition comprising a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof together with a pharmaceutically acceptable carrier or excipient.

15

The compounds and compositions according to the invention may be formulated for administration by any route, for example oral, topical or parenteral. The compositions may, for example, be made up in the form of tablets, capsules, powders, granules, lozenges, creams, syrups, sprays or liquid preparations, for example solutions or suspensions, which may be  
20 formulated for oral use or in sterile form for parenteral administration by injection or infusion.

Tablets and capsules for oral administration may be in unit dosage form, and may contain conventional excipients including, for example, binding agents, for example, syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example lactose, sugar,  
25 maize-starch, calcium phosphate, sorbitol or glycine; tableting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; and pharmaceutically acceptable wetting agents, for example sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice.

30

Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or another suitable vehicle before use. Such liquid preparations may contain conventional additives, including, for example, suspending agents, for example sorbitol,



methy1 cellulose, glucose syrup, gelatin, hydroxyethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, oily esters (for example glycerine), propylene glycol, or ethyl alcohol; 5 preservatives, for example methyl or propyl *p*-hydroxybenzoate or sorbic acid; and, if desired, conventional flavouring and colour agents.

Compositions according to the invention intended for topical administration may, for example, be in the form of ointments, creams, lotions, eye ointments, eye drops, ear drops, nose drops, 10 nasal sprays, impregnated dressings, and aerosols, and may contain appropriate conventional additives, including, for example, preservatives, solvents to assist drug penetration, and emollients in ointments and creams. Such topical formulations may also contain compatible conventional carriers, for example cream or ointment bases, ethanol or oleyl alcohol for lotions and aqueous bases for sprays. Such carriers may constitute from about 1% to about 15 98% by weight of the formulation; more usually they will constitute up to about 80% by weight of the formulation.

Compositions according to the invention intended for topical administration, in addition to the above, may also contain a steroidal anti-inflammatory agent; for example, betamethasone. 20

Compositions according to the invention may be formulated as suppositories, which may contain conventional suppository bases, for example cocoa-butter or other glycerides.

Compositions according to the invention intended for parenteral administration may 25 conveniently be in fluid unit dosage forms, which may be prepared utilizing the compound and a sterile vehicle, water being preferred. The compound, depending on the vehicle and concentration used, may be either suspended or dissolved in the vehicle. In preparing solutions, the compound may be dissolved in water for injection and filter-sterilised before being filled into a suitable vial or ampoule, which is then sealed. Advantageously, 30 conventional additives including, for example, local anaesthetics, preservatives, and buffering agents can be dissolved in the vehicle. In order to enhance the stability of the solution, the composition may be frozen after being filled into the vial, and the water removed under vacuum; the resulting dry lyophilised powder may then be sealed in the vial and a accompanying vial of water for injection may be supplied to reconstitute the liquid prior to

use. Parenteral suspensions may be prepared in substantially the same manner except that the compound is suspended in the vehicle instead of being dissolved and sterilisation cannot be accomplished by filtration. The compound may instead be sterilised by exposure to ethylene oxide before being suspended in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in such suspensions in order to facilitate uniform distribution of the compound.

A compound or composition according to the invention is suitably administered to the patient in an antimicrobially effective amount.

10

A composition according to the invention may suitably contain from 0.001% by weight, preferably (for other than spray compositions) from 10 to 60% by weight, of a compound according to the invention (based on the total weight of the composition), depending on the method of administration.

15

When the compositions according to the invention are presented in unit dosage form, for instance as a tablet, each unit dose may suitably comprise from 25 to 1000 mg, preferable from 50 to 500 mg, of a compound according to the invention.

20 Representative compositions of the present invention include those adapted for intranasal administration, in particular, those that will reach into the nasopharynx. Such compositions are preferably adapted for focussed delivery to, and residence within, the nasopharynx. The term 'focussed delivery' is used to mean that the composition is delivered to the nasopharynx, rather than remaining within the nares. The term 'residence' within the nasopharynx is used to mean that the composition, once delivered to the nasopharynx, remains within the nasopharynx over a course of several hours, rather than being washed away more or less immediately. Preferred compositions include spray compositions and creams. Representative spray compositions include aqueous compositions, as well as oily compositions that contain amphiphilic agents so that the composition increases in viscosity when in contact with moisture. Creams may also be used, especially creams having a rheology that allows the cream to spread readily in the nasopharynx.

25

30

Preferred aqueous spray compositions include, in addition to water, further excipients including a tonicity modifier such as a salt, for instance sodium chloride; preservative, such as

benzalkonium salt; a surfactant such as a non-ionic surfactant, for instance a polysorbate; and buffer, such as sodium dihydrogen phosphate; present in low levels, typically less than 1%. The pH of the composition may also be adjusted, for optimum stability of the drug substance during storage. For compounds of the present invention, a pH in the range 5 to 6, preferably about 5.3 to 5.8, typically about 5.5 is optimal.

Representative oily spray and cream compositions are described in WO 98/14189 (SmithKline Beecham). Representative aqueous sprays are described in International Application no PCT/GB98/03211 (SmithKline Beecham).

Suitably, the drug substance is present in compositions for nasal delivery in between 0.001 and 5%, preferably 0.005 and 3%, by weight of the composition. Suitable amounts include 0.5% and 1% by weight of the composition (for oily compositions and creams) and from 0.01 to 0.2% (aqueous compositions).

Spray compositions according to the present invention may be delivered to the nasal cavity by spray devices well known in the art for nasal sprays, for instance an air lift pump. Preferred devices include those that are metered to provide a unit volume of composition, preferably about 100µl, and optionally adapted for nasal administration by addition of a modified nozzle.

The invention is illustrated by the following Examples.

**Example 1 - 19,20-Dihydromutilin-14-[(5*R,S*)-3-phenyl-4,5-dihydroisoxazole-5-carboxylate]**

- Step 1. (3*R*) 3-Deoxo-11-deoxy-19,20-dihydro-3-methoxy-11-oxo-4-*epi*-mutilin - (3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin** (10.0g, 0.03 mole) in THF (250ml) was  
5 hydrogenated in the presence of 10% palladium on carbon (1.5g) at room temperature overnight. After filtration through celite, the filtrate was evaporated *in vacuo* to afford the title compound (9.75g, 97%) as a white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>) *inter alia* 0.85 (3H, t, J 7 Hz), 0.98 (3H, d, J 7 Hz), 3.08 (1H, q, J 13, 7 Hz), 3.22 (3H, s), 3.37-3.54 (1H, m), 4.55-4.66 (1H, m).
- Step 2. (3*R*) 3-Deoxo-11-deoxy-19,20-dihydro-3-methoxy-11-oxo-4-*epi*-mutilin-14-acrylate** - The product from Step 1 (9.75g, 0.029 mole) in dry dichloromethane (100 ml) was cooled to 0°C under argon. Triethylamine (16.1 ml, 0.116 mole) and  
4-dimethylaminopyridine (0.50g, 0.005 mole) were added, followed dropwise by acryloyl chloride (10.0 ml, 0.116 mole) and the mixture stirred overnight at room temperature. The  
15 solvent was removed *in vacuo* and the residue purified by chromatography eluting with 5% ethyl acetate/hexane to afford the title compound (5.7g, 50%) as a white solid; <sup>1</sup>H NMR (CDCl<sub>3</sub>) *inter alia* 0.70-0.86 (3H, m), 0.97-1.06 (3H, m), 2.42 (1H, dd, J 17, 12 Hz), 3.13-3.27 (4H, m), 3.40-3.54 (1H, m), 5.29-5.92 (2H, m), 6.02-6.28 (1H, m), 6.40 (1H, dd, J 17, 2 Hz).
- Step 3. (3*R*)-3-Deoxo-11-deoxy-19,20-dihydro-3-methoxy-11-oxo-4-*epi*-mutilin-14-[(5*R,S*)-3-phenyl-4,5-dihydroisoxazole-5-carboxylate** - The product of Step 2 (200 mg, 0.51 mmol) was dissolved in dioxane (5 ml) and dichloromethane (5 ml). α-Chlorobenzaldoxime {K. Lui, *J. Org. Chem.*, 1980, 45, 3916} (120 mg, 0.77 mmol) was added, followed by the addition of triethylamine (0.11 ml, 0.79 mmol) and the reaction mixture stirred for 5 days at room temperature. The mixture was diluted with dichloromethane, washed with saturated  
25 aqueous sodium hydrogen carbonate solution, dried (MgSO<sub>4</sub>) and concentrated *in vacuo*. The product was purified by chromatography on silica gel eluting with 1% ethyl acetate in dichloromethane. The title compound was isolated as a mixture of diastereoisomers (41 mg, 16%); <sup>1</sup>H NMR (CDCl<sub>3</sub>) *inter alia* 2.49 (1H, m), 3.12 (1H, m), 3.21 (3H, s), 3.43 (1H, m), 3.62 (1H, m), 5.08 (0.5H, dd, J 10.9, 7.6Hz), 5.14 (0.5H, dd, J 10.3, 7.5Hz), 5.88 (1H, m),  
30 7.40-7.52 (3H, m) and 7.65-7.71 (2H, m); MS(+ve ion electrospray) *m/z* 510 (MH<sup>+</sup>).
- Step 4. 19,20-Dihydromutilin-14-[(5*R,S*)-(3-phenyl-4,5-dihydroisoxazole-5-carboxylate)]** - The product of Step 3 (27 mg, 0.05 mmol) was dissolved in dioxane (1 ml) and treated with a saturated solution of zinc chloride in conc. HCl (1 ml). The reaction mixture was stirred at

room temperature for 1.5 hours and then diluted with ethyl acetate and a saturated aqueous solution of sodium hydrogen carbonate. The organic phase was washed with brine, dried (MgSO<sub>4</sub>) and concentrated *in vacuo*. The title compound was isolated as a mixture of diastereoisomers (20 mg, 76%); <sup>1</sup>H NMR (CDCl<sub>3</sub>) *inter alia* 3.38 (1H, m), 3.53 (2H, m),  
5 5.00 (0.5H, dd, J 11.1, 6.8Hz), 5.02 (0.5H, dd, J 10.6, 7.0Hz), 5.64 (1H, m), 7.30-7.39 (3H, m) and 7.56-7.63 (2H, m); MS(+ve ion electrospray) *m/z* 496 (MH<sup>+</sup>).

**Example 2 - 19,20-Dihydromutilin-14-[(5*R,S*)-3-(4-methoxyphenyl)-4,5-dihydroisoxazole-5-carboxylate]**

10 **Step 1. 19,20-Dihydromutilin-14-acrylate** - The product of Example 1, Step 2 (313 mg, 0.80 mmol) was dissolved in dioxane (4 ml) and treated with concentrated hydrochloric acid (3 ml). The reaction mixture was stirred at room temperature for 4 hours and then diluted with ethyl acetate and a saturated aqueous solution of sodium hydrogen carbonate. The organic phase was washed with brine, dried (MgSO<sub>4</sub>) and concentrated *in vacuo*. The product was purified  
15 by chromatography on silica gel eluting with 20% ethyl acetate in hexane. The title compound was isolated as a colourless foam (95 mg, 32%); <sup>1</sup>H NMR (CDCl<sub>3</sub>) *inter alia* 0.68 (3H, d, J 6.4Hz), 0.75 (3H, t, J 7.4Hz), 2.49 (1H, m), 3.46 (1H, m), 5.70 (1H, d, J 8.0Hz), 5.81 (1H, dd, J 10.3, 1.5Hz), 6.08 (1H, dd, J 17.2, 10.3Hz), 6.35 (1H, dd, J 17.2, 1.5Hz).

**Step 2. 19,20-Dihydromutilin-14-[(5*R,S*)-3-(4-methoxyphenyl)-4,5-dihydroisoxazole-5-carboxylate]** - The product of Step 1 (90 mg, 0.24 mmol) was dissolved in dioxane (4 ml) and dichloromethane (4 ml). α-Chloro-(4-methoxybenzaloxime) {K. Lui, *J. Org. Chem.*, 1980, 45, 3916} (67 mg, 0.36 mmol) was added, followed by the addition of triethylamine (0.05ml, 0.36 mmol) and the reaction mixture stirred for 2 hours at room temperature. The mixture was diluted with dichloromethane, washed with saturated aqueous sodium hydrogen carbonate  
25 solution, dried (MgSO<sub>4</sub>) and concentrated *in vacuo*. The product was purified by chromatography on silica gel eluting with 5% ethyl acetate in dichloromethane. The title compound was isolated as a mixture of diastereoisomers (89 mg, 71%); <sup>1</sup>H NMR (CDCl<sub>3</sub>) *inter alia* 0.70 (3H, d, J 7.0Hz), 0.92 (3H, d, J 7.0Hz), 3.40 (1H, m), 3.83 (3H, s), 5.01 (0.5H, dd, J 11.0, 6.7Hz), 5.09 (0.5H, dd, J 10.8, 6.8Hz), 5.69 (1H, m), 6.90 (2H, d, J 8.5Hz), 7.59  
30 (1H, d, J 8.5Hz), 7.63 (1H, d, J 8.5Hz); MS(+ve ion electrospray) *m/z* 526 (MH<sup>+</sup>).

**Example 3 - 19,20-Dihydromutilin-14-[(5*R,S*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate]** **Step 1. 1-Aza-bicyclo[2.2.2]oct-4-yl-carbaldehyde oxime**

**hydrochloride** - A solution of quinuclidin-4-carbaldehyde (E. Ceppi and C.A. Grob, *Helv. Chim. Acta.*, 1974, 57, 2332) (175mg, 1.25mmol) and hydroxylamine hydrochloride (88mg, 1.25mmol) in methanol (5ml) was stirred at room temperature for 18 hours. The solvent was evaporated and the residue partitioned between chloroform and saturated potassium carbonate solution. The organic layer was separated, dried (MgSO<sub>4</sub>) and concentrated giving the title compound as a gum (165mg, 86%); MS (electrospray) *m/z* 155 (MH<sup>+</sup>).

The product was suspended in diethyl ether (5ml) at 5°C and was treated with a 1M solution of hydrogen chloride in diethyl ether (1ml). After stirring at 5°C for 10 minutes the mixture was evaporated giving the title compound (190mg) which was used immediately in the next reaction.

**Step 2. (3*R*)-3-Deoxo-11-deoxy-19,20-dihydro-3-methoxy-11-oxo-4-*epi*-mutilin-14-[(5*R,S*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate]** - A suspension of the product of Step 1 (190mg, 1mmol) in N,N-dimethylformamide (10ml) was stirred at room temperature under an atmosphere of argon. N-Bromosuccinimide (196mg, 1.1mmol) was added portion-wise and the mixture was stirred at room temperature for 90 minutes. The yellow suspension was cooled to -10°C and a solution of (3*R*)-3-deoxo-11-deoxy-19,20-dihydro-3-methoxy-11-oxo-4-*epi*-mutilin-14-acrylate (390mg, 1mmol) in N,N-dimethylformamide (2ml) was added. A solution of triethylamine (0.28ml, 2mmol) in N,N-dimethylformamide (2ml) was then added dropwise over 30 minutes. The mixture was stirred at room temperature for 90 minutes and the solvent was evaporated. The residue was partitioned between chloroform and saturated sodium carbonate solution. The chloroform layer was separated, washed with brine, dried (MgSO<sub>4</sub>) and concentrated. Purification of the residue by chromatography on silica gel eluting with chloroform-methanol-35% ammonia solution (10:1:0.1) gave the title compound as a 1:1 mixture of diastereoisomers as a colourless solid (280mg, 52%); MS (electrospray) *m/z* 543 (MH<sup>+</sup>).

**Step 3. 19,20-Dihydromutilin-14-[(5*R,S*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate]** - A solution of the product of Step 2 (250mg, 0.46mmol) in dioxane (3ml) and concentrated hydrochloric acid (3ml) was stirred at room temperature under an atmosphere of argon for 4 hours. The solution was diluted with ethyl acetate and water. The aqueous layer was separated and solid potassium carbonate was added to adjust the pH of the solution to pH 10. The solution was extracted with chloroform, the chloroform layer was separated, washed with brine, dried (MgSO<sub>4</sub>) and concentrated. The residue was suspended in acetone (5ml) and the mixture was stirred at room temperature for 15 minutes, the solid was

filtered, washed with diethyl ether and dried giving the title compound as a 1:1 mixture of diastereoisomers as a colourless solid (180mg, 74%);  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) *inter alia* 0.65-0.77 (6H, m), 0.92-0.96 (6H, m), 2.96 (6H, t, J 7.5Hz), 3.05-3.25 (2H, m), 3.35 -3.45 (1H, m), 4.83 (0.5H, dd, J 9.9, 5.5Hz), 4.90 (0.5H, J 9.0, 7.5Hz), 5.66 (1H, d, J 8.1Hz); MS (electrospray)  $m/z$  529 ( $\text{MH}^+$ ).

**Example 4 and 5 - 19,20-Dihydromutilin-14-[(5R)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate] and 19,20-dihydromutilin-14-[(5S)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate].** The product from Example 3 (50mg) was purified by HPLC using a Hypersil BDS Hypercarb column (100 × 3 mm, 7mm), eluting with acetonitrile : ethanol : 37% aqueous ammonia (800:200:1) at a flow rate of 10 ml /minute. Other parameters were as follows: detection wavelength, 210 nm; injection volume; 0.1ml. The material was purified in several chromatography runs and gave samples of the title compounds (5mg and 7.5mg respectively); MS (electrospray)  $m/z$  529 ( $\text{MH}^+$ ). The later compound was found to contain 10% of former. Absolute stereochemistry in each case was not determined.

**Example 6 - Mutilin-14-[(5R,S)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate].**

**Step 1. Mutilin 11-trifluoroacetate -** Mutilin (960 mg) in dry tetrahydrofuran (12 ml) was treated with pyridine (0.3 ml) and the solution was cooled to 0°C. Trifluoroacetic anhydride (0.48 ml) was added dropwise over 3 minutes to the stirred solution. The solution was kept at 0°C for 2 hours, and was then diluted with ethyl acetate (100 ml) and washed with water (2 x 30 ml), sodium bicarbonate solution (30 ml), and saturated sodium chloride solution (30 ml). The solution was dried (sodium sulphate) and the solvent was evaporated under reduced pressure to give a colourless gum. The gum was chromatographed on silica gel using 1:9 to 1:4 ethyl acetate/ hexane to give the title compound as colourless crystals (570 mg).

Recrystallisation from dichloromethane/ hexane gave colourless rods, m.p. 170 - 171 °C;  $\nu_{\text{max}}$  ( $\text{CHCl}_3$ ) 3636, 1777, and 1736  $\text{cm}^{-1}$ ; MS(EI)  $m/z$  416 ( $\text{M}^+$ ).

**Step 2. Mutilin 14-acrylate-11-trifluoroacetate -** Mutilin 11-trifluoroacetate (Step 2) (3.0 g, 0.0072 mole), triethylamine (3.74 g, 0.037 mole) and a catalytic amount of 4-dimethylaminopyridine in dichloromethane (100 ml) was treated with acryloyl chloride (3.33 g, 0.037 mole) overnight at room temperature under argon. The reaction mixture was

partitioned between water and dichloromethane. The organic layer was dried over magnesium sulfate and the solvents removed *in vacuo*. Chromatography of the residue on silica gel eluting with ethyl acetate / petroleum ether 40-60° (1:10) provided the title compound 1.25 g (37%); <sup>1</sup>H NMR (CDCl<sub>3</sub>) *inter alia* 0.69 (3H, d, J 6.6 Hz), 0.84 (3H, d, J 7 Hz), 1.06 (3H, s), 1.52 (3H, s), 2.1 to 2.4 (4H, m), 2.65 (1H, m), 5.0 (1H, d, 6.9 Hz), 5.20-5.37 (2H, m), 5.72-5.86 (2H, m), 6.0-6.1 (1H, m), 6.3-6.5 (2H, m).

**Step 3. Mutilin-14-[(5*R,S*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate]-11-trifluoroacetate** - The product of Example 3, Step 1 (950mg, 4.2 mmol) was suspended in N,N-dimethylformamide (70 ml) and cooled to 0 °C under an argon atmosphere. N-bromosuccinimide (826 mg, 4.6 mmol) was then added portionwise over 20 minutes. The yellow suspension was allowed to warm to room temperature and then stirred for a further 90 minutes. The reaction was then cooled to -10°C and mutilin-14-acrylate-11-trifluoroacetate (1.98 g, 4.2 mmol) (Step 2) was added followed by the dropwise addition of triethylamine (1.17 ml, 8.4 mmol) over a 20 minute period. The reaction was stirred for 1 hour at -10 °C and for a further 2 hours at room temperature after which the solvent was removed *in vacuo*. The residue was partitioned between saturated potassium carbonate and chloroform. The aqueous layer was then extracted with a further 2 portions of chloroform and the combined organic fraction dried (sodium sulphate), filtered and evaporated *in vacuo*. The product was purified by silica gel chromatography eluting with chloroform: methanol : 35% aqueous ammonia (94 : 6 : 0.5) to give the title compound (1.8 g, 67%); MS (+ve ion electrospray) *m/z* 623 [M+H]<sup>+</sup>.

**Step 4. Mutilin-14-[(5*R,S*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate]** - A solution of the product from Step 1 in tetrahydrofuran/water (5:1) was treated with 0.5M aqueous sodium hydroxide (5.78 ml) and allowed to stand for 1 hour. The mixture was then partitioned between chloroform and saturated aqueous potassium carbonate. The aqueous layer was separated and further extracted with 2 portions of chloroform. The combined organic fraction was dried (sodium sulphate), filtered and concentrated. Purification by silica gel chromatography eluting with chloroform: methanol : 35% aqueous ammonia (10 : 1 : 0.1) to give the title compound (509 mg, 98%) as a white foam; MS (+ve ion electrospray) *m/z* 527 [M+H]<sup>+</sup>.

**Example 7 and 8 - Mutilin-14-[(5*S*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate] (7) and mutilin-14-[(5*R*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate] (8).** The product from Example 6, Step 4, was crystallised



from chloroform/pentane to give 509mg of crystalline material. Recrystallisation from methanol then gave Example 7 (178mg) as colourless crystals; MS (+ve ion electrospray)  $m/z$  527  $[M+H]^+$ . Absolute stereochemistry was determined by X-ray crystallography.

5 The mother liquor from the first crystallisation was concentrated *in vacuo* and the residue again dissolved in chloroform/pentane from which a small amount of crystalline material appeared. The solution was filtered, concentrated *in vacuo*, re-dissolved in methanol and filtered again. The methanol solution was concentrated *in vacuo* to give a white solid which was recrystallised from methanol/water to give Example 8 (160mg) as pale yellow prismatic crystals; MS (+ve ion electrospray)  $m/z$  527  $[M+H]^+$ .

10

**Example 9 - Mutilin-14-[(5*R,S*)-3-(1-methyl-piperidin-4-yl)-4,5-dihydroisoxazole-5-carboxylate].**

**Step 1. 1-Methyl-piperidin-4-carbaldehyde oxime** - 1-Methyl piperidin-4-carbaldehyde {K. Prokai-Tatrai, *J. Organomet. Chem.*, 1986, 315, 231} (880mg, 7mmol) was dissolved in  
15 methanol (20 ml) and stirred with hydroxylamine hydrochloride (480mg) for 16 hours. The solvent was removed and the residue partitioned between saturated aqueous potassium carbonate and chloroform. The aqueous layer was separated and extracted with a further 2 portions of chloroform. The combined organic fraction was dried (sodium sulphate), filtered and condensed to give the title compound as a white crystalline solid (663mg 67%); MS (+ve ion electrospray)  $m/z$  143  $[M+H]^+$ .

20

**Step 2. Mutilin-14-[(5*R,S*)-3-(1-methyl-piperidin-4-yl)-4,5-dihydroisoxazole-5-carboxylate]** - The product from Step 1 (623mg, 4.42 mmol) was treated with 1 M hydrogen chloride in diethyl ether and then evaporated to dryness. The product was then dissolved in dimethylformamide (60 ml), and treated successively with N-bromosuccinimide (865 mg, 4.86  
25 mmol), mutilin-14-acrylate-11-trifluoroacetate (2.28 g, 4.86 mmole) and triethylamine (1.23 ml, 8.84 mmol) according to the procedure in Example 6, Step 3. The product was purified by silica gel chromatography eluting with chloroform : methanol : 35% aqueous ammonia (89.8 : 10 : 0.2) to give the title compound as a foam (900mg, 37%); MS (+ve ion electrospray)  $m/z$  515  $[M+H]^+$ .

30

**Example 10 - Mutilin-14-[(5*R,S*)-3-(*exo*-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-4,5-dihydroisoxazole-5-carboxylate].**

**Step 1. *exo*-8-Methyl-8-aza-bicyclo[3.2.1]oct-3-yl-carbaldehyde oxime** - To *exo*-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl-nitrile (*J. Am. Chem. Soc.*, 1960, 82, 151) (7.28 g, 0.048 mole) in

toluene (200 ml), was added diisobutylaluminium hydride (1.5 M in toluene, 42 ml, 0.063 mole) dropwise. After 3 h, the reaction mixture was poured into 2 M hydrochloric acid at -20 °C and stirred for 2 h. The aqueous layer was isolated and saturated with potassium carbonate. The resulting solution was then extracted with chloroform. The organic fractions were dried (sodium sulphate), filtered and concentrated *in vacuo* to give a brown oil.

The product was then treated with hydroxylamine hydrochloride (1.81 g, 0.026 mole) as described in Example 9, Step 1. The crude product was recrystallised from ethyl acetate to give needles (1.72 g, 21%); MS (+ve ion electrospray)  $m/z$  169 [M+H]<sup>+</sup>.

**Step 2. Mutilin-14-[(5*R,S*)-3-(*exo*-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-4,5-**

**dihydroisoxazole-5-carboxylate]-11-trifluoroacetate.** - The product from Step 1 (1.44 g, 0.00306 mole) was treated successively with 1M hydrochloric acid in diethyl ether, *N*-bromosuccinimide, mutilin-14-acrylate-11-trifluoroacetate and triethylamine according to the procedure in Example 6, Step 3. The product was purified by silica gel chromatography eluting with chloroform : methanol : 35% aqueous ammonia (93.4 : 6 : 0.6) to give the title compound (1.18 g, 60%) as a foam; MS (+ve ion electrospray)  $m/z$  637 [M+H]<sup>+</sup>.

**Step 3. Mutilin-14-[(5*R,S*)-3-(*exo*-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-4,5-**

**dihydroisoxazole-5-carboxylate]** - The product from Step 2 was treated with sodium hydroxide as described in Example 6, Step 2. The residue was purified as described in Example 10, Step 2 to give the title compound (700mg, 65%) as a foam; MS (+ve ion electrospray)  $m/z$  541 [M+H]<sup>+</sup>.

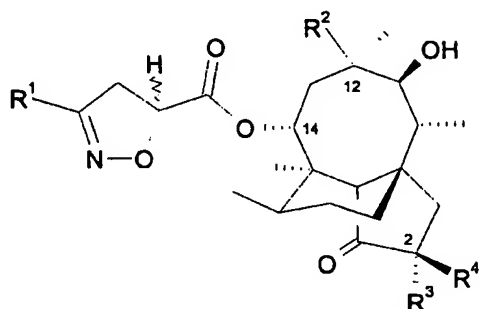
### Biological Data

Compounds of the present invention were assessed for anti-bacterial activity in a conventional MIC assay against a range of pathogenic organisms.

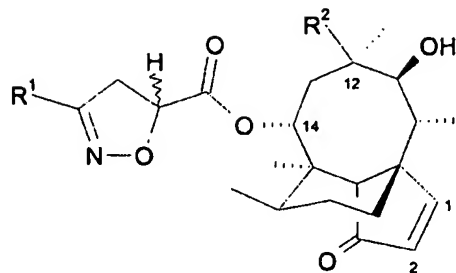
Compounds were found to have MICs in the range 0.06 to 4 µg/ml against *Staph aureus* Oxford and 0.06 to 4 µg/ml against *Strep pneumoniae* (R6).

# Claims

1. A compound of Formula (IA) or (IB):



(IA)



(IB)

in which:

R<sup>1</sup> is an optionally substituted aryl group, or an optionally substituted nitrogen containing heterocycle;

R<sup>2</sup> is vinyl or ethyl;

- 10 R<sup>3</sup> is H, OH or F; and R<sup>4</sup> is H, or R<sup>3</sup> is H and R<sup>4</sup> is F.

2. A compound of formula (I) as claimed in claim 1 in which R<sup>1</sup> is a phenyl group substituted by up to three substituents.

- 15 3. A compound of formula (I) as claimed in claim 1 in which R<sup>1</sup> is optionally substituted azabicyclo-octyl and piperidinyl.

4. A compound of formula (I) as claimed in claim 3 in which R<sup>1</sup> is selected from optionally substituted 1-aza-bicyclo[2.2.2]oct-4-yl, piperidin-4-yl and 8-aza-bicyclo[3.2.1]oct-3-yl.

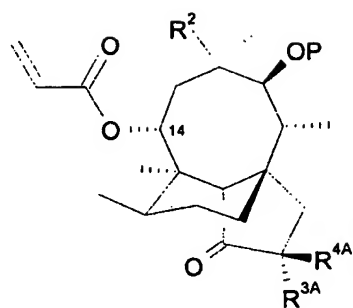
20

5. A compound of formula (I) as claimed in claim 3 or 4 in which a substituent is selected from alkyl, alkyloxy, alkenyl and alkenyloxy, each of which may be carried by either a bridgehead or a non-bridgehead carbon atom, and, for a nitrogen atom, oxygen, to form an N-oxide, or mono- or di(C<sub>1-6</sub>)alkyl.

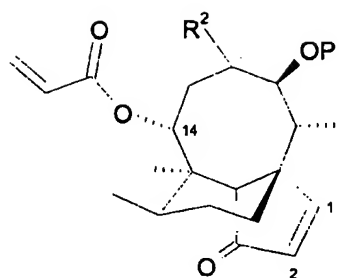
25

6. A compound of formula (I) as claimed in claim 1 selected from the compounds described in the main titles of Examples 1 to 10.

7. A compound of formula (I) as claimed in 6 selected from:  
19,20-Dihydromutilin-14-[(5*R,S*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate];
- 5 19,20-Dihydromutilin-14-[(5*R*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate];  
19,20-Dihydromutilin-14-[(5*S*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate];  
Mutilin-14-[(5*R,S*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate];
- 10 Mutilin-14-[(5*S*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate];  
Mutilin-14-[(5*R,S*)-3-(1-aza-bicyclo[2.2.2]oct-4-yl)-4,5-dihydroisoxazole-5-carboxylate];  
Mutilin-14-[(5*R,S*)-3-(1-methyl-piperidin-4-yl)-4,5-dihydroisoxazole-5-carboxylate], and  
Mutilin-14-[(5*R,S*)-3-(*exo*-8-methyl-8-aza-bicyclo[3.2.1]oct-3-yl)-4,5-dihydroisoxazole-5-carboxylate].
- 15
8. A pharmaceutical composition comprising a compound as claimed in claim 1, or a pharmaceutically acceptable salt or derivative thereof, and a pharmaceutically acceptable carrier.
- 20 9. A compound of formula (I) as claimed in claim 1 for use in therapy.
10. A method of treating microbial infections in animals which method comprises administering an effective amount of a compound of formula (I) as claimed in claim 1 to a patient in need thereof.
- 25
11. A process for preparing a compound of formula (I) as claimed in claim 1 which process comprises:
- (a) reacting a compound of formula (IIA) or (IIB):



(IIA)



(IIB)

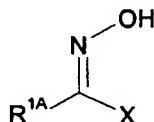
in which:

P is hydrogen or a removable hydroxy-protecting group;

- 5 R<sup>3A</sup> and R<sup>4A</sup> are R<sup>3</sup> and R<sup>4</sup> as defined in claim 1 for formulae (IA) and (IB) or a group convertible to R<sup>3</sup> and R<sup>4</sup> respectively; and

R<sup>2</sup> is as defined in claim 1;

with a compound of formula (III):



(III)

in which:

R<sup>1A</sup> is R<sup>1</sup> as defined for formulae (IA) and (IB) or a group convertible to R<sup>1</sup>; and

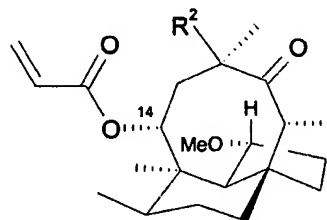
X is chloro or bromo, or a group convertible to chloro or bromo;

in a 1,3-dipolar cycloaddition reaction and thereafter, and if so needed;

- 15 converting P to hydrogen, and if necessary

converting an R<sup>3A</sup> or R<sup>4A</sup> group to an R<sup>3</sup> or R<sup>4</sup> group; or

(b) for a compound of formula (IA) in which R<sup>3</sup> and R<sup>4</sup> are both hydrogen, reacting an *epi*-mutilin compound of formula (IIC):



(IIC)

wherein  $R^2$  is as hereinbefore defined;

with a compound (III), as hereinbefore defined;

and then treating the product with an acid;

- 5 and where required or desired converting an  $R^{1A}$  group to an  $R^1$  group.

## INTERNATIONAL SEARCH REPORT

Internatio Application No

PCT/EP 00/04232

**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC 7 C07D261/04 A61K31/42

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07D A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, CHEM ABS Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99 21855 A (SANDERSON FRANCIS DOMINIC ;DABBS STEPHEN (GB); HUNT ERIC (GB); FRY) 6 May 1999 (1999-05-06) cited in the application the whole document	1-11
A	WO 97 25309 A (HUNT ERIC ;HINKS JEREMY DAVID (GB); SMITHKLINE BEECHAM PLC (GB); T) 17 July 1997 (1997-07-17) cited in the application the whole document	1-11
A	FR 2 157 828 A (SANDOZ SA) 8 June 1973 (1973-06-08) claims	1-11
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
- \*L\* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- \*O\* document referring to an oral disclosure, use, exhibition or other means
- \*P\* document published prior to the international filing date but later than the priority date claimed

\*T\* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

\*X\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

\*Y\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*G\* document member of the same patent family

Date of the actual completion of the international search

4 September 2000

Date of mailing of the international search report

12/09/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
 NL - 2280 HV Rijswijk  
 Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
 Fax: (+31-70) 340-3016

Authorized officer

Frelon, D

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/04232

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 013 768 A (SANDOZ AG) 6 August 1980 (1980-08-06) abstract	1-11
A	EGGER H ET AL: "NEW PLEUROMUTILIN DERIVATIVES WITH ENHANCED ANTIMICROBIAL ACTIVITY.II. STRUCTURE-ACTIVITY CORRELATIONS" JOURNAL OF ANTIBIOTICS,JP,JAPAN ANTIBIOTICS RESEARCH ASSOCIATION. TOKYO, vol. 29, no. 9, 1 September 1976 (1976-09-01), pages 923-927, XP002028938 ISSN: 0021-8820 the whole document	1-11



# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/04232

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9921855 A	06-05-1999	AU 9636198 A EP 1028961 A NO 20002173 A	17-05-1999 23-08-2000 05-06-2000
WO 9725309 A	17-07-1997	AU 715229 B AU 1307897 A BG 102600 A BR 9612426 A CA 2240467 A CN 1214039 A CZ 9802124 A EP 0874809 A HU 9900973 A JP 2000503642 T NO 983074 A PL 327737 A SK 91098 A US 6020368 A ZA 9700017 A AU 4203697 A BR 9711008 A CN 1231665 A CZ 9900293 A WO 9805659 A EP 0934316 A NO 990463 A PL 331470 A ZA 9706817 A	20-01-2000 01-08-1997 30-09-1999 13-07-1999 17-07-1997 14-04-1999 16-12-1998 04-11-1998 30-08-1999 28-03-2000 31-08-1998 21-12-1998 11-02-1999 01-02-2000 02-07-1998 25-02-1998 17-08-1999 13-10-1999 16-06-1999 12-02-1998 11-08-1999 01-02-1999 19-07-1999 01-02-1999
FR 2157828 A	08-06-1973	CH 572893 A CH 568272 A CH 575375 A CH 560180 A CH 559719 A AT 335054 B AT 845872 A BE 789629 A BG 21386 A BG 20773 A CA 979447 A CS 219306 B CY 987 A DD 100706 A DE 2248237 A DE 2265666 C DK 139720 B DK 498076 A,B, DK 498176 A,B, FI 58635 B FI 794060 A,B, FI 794061 A,B, GB 1410506 A GB 1410505 A HK 25379 A HU 168436 B IE 37699 B IE 37700 B IL 40494 A	27-02-1976 31-10-1975 14-05-1976 27-03-1975 14-03-1975 25-02-1977 15-06-1976 03-04-1973 20-05-1976 20-12-1975 09-12-1975 25-03-1983 02-08-1979 05-10-1973 12-04-1973 03-02-1983 02-04-1979 03-11-1976 03-11-1976 28-11-1980 27-12-1979 27-12-1979 15-10-1975 15-10-1975 27-04-1979 28-04-1976 28-09-1977 28-09-1977 30-01-1976

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/04232

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
FR 2157828 A		IL 47262 A	30-01-1976
		JP 48044244 A	26-06-1973
		JP 57007147 B	09-02-1982
		MY 10379 A	31-12-1979
		NL 7213254 A,B,	09-04-1973
		NO 136459 B	31-05-1977
		NO 136837 B	08-08-1977
		NO 137440 B	21-11-1977
		OA 4192 A	31-12-1979
		PH 11870 A	04-08-1978
		RO 67478 A	15-03-1980
		RO 67479 A	21-06-1981
		SE 398348 B	19-12-1977
		SE 424074 B	28-06-1982
		SE 7509456 A	26-08-1975
		SE 419984 B	07-09-1981
		SE 7509457 A	26-08-1975
		US 4278674 A	14-07-1981
		US 4107164 A	15-08-1978
		US 4208326 A	17-06-1980
		YU 249772 A	27-04-1983
EP 0013768 A	06-08-1980	AT 6062 T	15-02-1984
		AU 533691 B	08-12-1983
		AU 5453580 A	17-07-1980
		CA 1125778 A	15-06-1982
		CY 1353 A	24-04-1987
		DE 2966635 D	08-03-1984
		DK 4280 A,B,	13-07-1980
		ES 487584 D	16-02-1981
		ES 8101536 A	16-03-1981
		FI 800024 A,B,	13-07-1980
		HK 40587 A	29-05-1987
		IE 49356 B	18-09-1985
		IL 59108 A	31-07-1983
		JP 55094359 A	17-07-1980
		KE 3689 A	20-02-1987
		MY 72687 A	31-12-1987
		NZ 192576 A	31-05-1984
		PH 17391 A	08-08-1984
		PT 70682 A	01-02-1980
		US 4428953 A	31-01-1984
		YU 6280 A	28-02-1983
		ZA 8000175 A	26-08-1981

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☒ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☒ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**